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TITLE: Predictive Value of Serum Organochlorine Levels and  
Breast Cancer in Occupationally Exposed Populations

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## Introduction

The role of organochlorine compounds in breast cancer is still uncertain. It is not known whether estrogens act as initiators, promoters, or both with regard to breast cancer. Moreover, the diversity of estrogenic chemicals is quite large and estrogenic response in breast tissues is a complex process that is yet to be elucidated. However despite these unknowns, the circumstantial evidence that estrogens and estrogen-like compounds in some way contribute to risk for breast cancer is compelling. Our study has the potential to contribute useful information to the questions of the role of estrogenic agents in breast cancer risk by evaluating separately and simultaneously more than 30 organochlorine compounds, some of which are estrogenic and some are antiestrogenic. We had originally planned to conduct two related case-control studies, one evaluating estrogenic pesticides and PCBs and the other evaluating dioxins, furans, and PCBs that have antiestrogenic properties. Now, for a variety of reasons, primarily because it is a stronger design, we hope to conduct the study in one case-control design where both estrogenic and antiestrogen compounds will be evaluated in the same subjects.

## Body

During the reporting period the following activities have been performed:

**1) Selection of specimens** - The selection strategy for specimens (cases and controls) was applied to the Janus Serum Bank population and the study specimens (150 cases and 150 controls) were identified and delivered by Dr. Jellum to the laboratory in Atlanta. The specimens arrived still frozen and in good condition. The specimens were secured in a freezer.

**2) Enhancement and testing of the analytical method** - Earlier this year, we completed a feasibility study using a set of 16 thawed Janus serum samples. Since then, we have continued in the method development phase of this study to validate a method to allow the analysis of 75 congeners of PCDDs, PCDFs, cPCBs, PCBs, and persistent pesticides in one milliliter of serum. These analytes are quantified by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC-IDHRMS), the most accurate and precise method of quantitation.

We have received and tested a new automated sample cleanup system which will allow the sample throughput necessary to complete the laboratory analyses by the end of February 1997, as originally agreed upon. Along with a new solid-phase extraction, this new automated multi-column cleanup procedure has been tested for one milliliter of serum samples and shown to produce acceptable results.

The very low levels of many of the congeners in one milliliter of normal human serum required that we remake and validate all the analytical standards necessary to calibrate the HRMS for IDMS quantification. IDMS stands for 'isotope-dilution mass

spectrometry', the technique used for quantitation where the ratio of native (naturally occurring compounds) and their carbon-13 labeled internal standard are compared. IDHRMS is IDMS performed on a HRMS (as opposed to LRMS - low resolution MS). All of these standards and carbon-13 labeled internal standards for IDMS have now been prepared and aliquoted.

The feasibility study of 16 representative Janus samples was invaluable in defining the range of levels that we would find in the Janus study and thus appropriately make our standards to cover the range of expected levels. This information was also used to help set levels of the various congeners in quality control materials. Looking back at the 16 samples in the feasibility study we can say that some PCBs and pesticides were found in all samples, but not all proposed analytes were found in every sample. In general all PCDDs/PCDFs were essentially at the detection limit, however we did observe coplanar PCBs in some samples. We did note a trend that the concentration of analytes in that the most recently collected samples were generally lower than those collected in the 1970s (that means we should be able to see a difference in the concentration of some of the analytes in samples from the same person collected years apart).

Currently we are developing calibration and QA/QC criteria which will be used to insure the reliability of the results as outlined in our laboratory certification under the Clinical Laboratory Improvement Act (CLIA).

We have recently added a third high resolution mass spectrometer in order to expedite the laboratory throughput for the study. The most sensitive HRMS (Micromass's AutoSpec ULTIMA) will be used for the PCDDs, PCDFs and cPCBs. A Micromass AutoSpec and 70SE HRMS will be used for the pesticides and ortho-substituted PCB congeners, respectively. This summer we also completed an upgrade and subsequent validation of new software for the 70SE, to improve the sensitivity and stability of the mass spectrometer.

Finally, we have completely automated the data acquisition and data transfer for the HRMS's to a data base for QA/QC, as well as all aspects of data handling and reporting.

**3) Development of a detailed statistical analysis plan** - Work was begun on a detailed plan for the statistical analysis of the data. This plan will stipulate the exact statistical analyses and comparisons that will be conducted. The plan will be consistent with the general analyses in the original protocol.

## **Conclusion**

The efforts of this past year demonstrate that the approaches selected will work and should yield useful data. The study is proceeding on all fronts and we still expect to finish on schedule.

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